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## Article

# Common Genes Involved in Autophagy, Cellular Senescence and the Inflammatory Response in AMD and Drug Discovery Identified via Biomedical Databases

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Purpose: Retinal pigment epithelial cell autophagy dysfunction, cellular senescence, and the retinal inflammatory response are key pathogenic factors in age-related macular degeneration (AMD), which has been reviewed in our previously work in 2019. This study aims to identify genes collectively involved in these three biological processes and target drugs in AMD.

Methods: The pubmed2ensembl database was used to perform text mining. The GeneCodis database was applied to analyze gene ontology biological process and the KEGG pathway. The STRING database was used to analyze protein-protein interaction analysis and hub genes were identified by the Cytoscape software. The Drug Gene Interaction Database was used to perform drug-gene interactions.

Results: We identified 62 genes collectively involved in AMD, autophagy, cellular senescence, and inflammatory response, 19 biological processes including 42 genes, 11 enriched KEGG pathways including 37 genes, and 12 hub genes step by step via the above biomedical databases. Finally, five hub genes (IL-6, VEGF-A, TP53, IL-1 $\beta$ , and transforming growth factor [TGF]- $\beta$ 1) and their specific interaction modes were identified, corresponding with 24 target drugs with therapeutic potential for AMD.

**Conclusions:** IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1 are pivotal in autophagy, cellular senescence, and the inflammatory response in AMD, corresponding with 24 drugs with therapeutic potential for AMD, providing definite molecular mechanisms for further research and new possibilities for AMD treatment in the future.

**Translational Relevance:** IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1 may be new targets for AMD gene therapy and drug development.

# Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in elderly people around the world.<sup>1</sup> Globally, the projected number of people with AMD will increase to 288 million in 2040.<sup>2</sup> Approximately 67 million people in Europe are currently affected by AMD and this number is expected to increase to 77 million by 2050.<sup>3</sup> The cost

of visual impairment owing to AMD accounts for US\$343 billion or 12% of the total global cost of visual impairment,<sup>4</sup> revealing that AMD is becoming a main public health issue. There are two forms of AMD: atrophic (dry) and neovascular (wet) AMD, accounting for 90% and 10% of all cases, respectively.<sup>5–7</sup> Intravitreal injection of anti-vascular endothelial cell growth factor (VEGF) drugs has become a well-recognized therapy for patients with wet AMD. Nevertheless, because AMD is a disease caused by

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multiple factors, even after this progress, therapy is far from being perfect and there remains ample room for improvement. Although some approaches have been evaluated for dry AMD, such as neuroprotection, visual cycle modulation, inflammation inhibition, cell-based therapy, and complement suppression, an effective therapeutic method remains elusive.<sup>8</sup> The current management of dry AMD basically depends on early recognition of changes in visual function and detection of choroidal neovascularization.<sup>7</sup> Some researchers advocate that future directions in dry AMD research should emphasize systems biology approaches that integrate -omic, pharmacologic, and clinical data based on designing computer models to predict disease progression, and identify biomarkers and mechanisms, and so on.<sup>6</sup> Autophagy dysfunction and cellular senescence of retinal pigment epithelium (RPE) cells and abnormal inflammatory responses in the retina are important pathologic factors in AMD, which has been reviewed in our previously work in 2019. These factors stimulate and restrict each other, eventually leading to vision loss.<sup>9</sup> Hence, if we can identify genes that are jointly involved in autophagy and cellular senescence of RPE cells and the retinal inflammatory response and then apply drugs to target these genes, it may be possible to comprehensively achieve the effects of antineovascularization, autophagy improvement, cellular senescence alleviation, and inhibitions of abnormal inflammatory responses to delay the progression of AMD and obtain better clinical efficacy.

Text mining is a powerful technique to quickly extract critical information from a large amount of the biomedical literature<sup>10,11</sup> to analyze and summarize the data. When queries are performed, text mining extracts all of the genes related to the search concepts from the biological literature available, which can be used for the subsequent analyses, providing links between the literature and genes for data exploration and providing valuable information about the relationships between diverse diseases.<sup>12</sup>

Gene ontology (GO) analysis is a powerful biologic tool that uses a defined nomenclature to annotate genes/proteins within three categories: "molecular function," "biological process," and "cellular component," assisting in uncovering functional mechanisms in proteomic, transcriptomic and genomic data.<sup>13</sup> A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis can present the whole pathways relevant to signal transduction and other biological processes<sup>14</sup> to discover target genes and their target regulatory factors. Both GO and KEGG pathways belong to enrichment analysis, which is an important tool for annotating functions of genes and analyzing data. The enrichment analysis can be understood as a process of reflecting fragmented information through a whole. After an amount of unique genes related to the disease are obtained through text mining, GO and KEGG pathway analyses are often used in combination<sup>15</sup> to reflect gene functions and identify enriched genes relevant to the disease, which greatly facilitates some forms of automated knowledge extraction, such as text mining.<sup>16</sup>

Because GO terms are made up of a pure set of genes, whereas the KEGG pathway involves proteins, a pathway analysis requires further verification of protein functions. Protein–protein interaction (PPI) extraction through biological literature curation is widely used for proteome analysis.<sup>17</sup> Most proteins perform their functions through their interactions,<sup>18</sup> which can be present in the PPI network. In addition, PPI can identify hub genes or proteins and aid the identification of drug targets.<sup>18,19</sup>

The Drug Gene Interaction Database (DGIdb) collects and summarizes gene druggability information and drug–gene interactions from web resources, databases, and papers.<sup>20</sup> Although developing new drugs and verifying their therapeutic effects may take a long time, repositioning existing drugs based on drug–gene interactions may uncover previously unknown abilities to treat other diseases at a lower cost. Sildenafil (Viagra), which was used for angina treatment, but was later found to be effective in treating erectile dysfunction, is the typical example of drug reuse.<sup>21,22</sup>

In this study, genes jointly involved in autophagy, cellular senescence, and the inflammatory response in AMD were identified by text mining. GO biological process and KEGG pathway analyses were performed to identify enriched biological processes and genes relevant to AMD. A PPI analysis was further used to assess the impact of enriched genes on the PPI network and determine hub genes, exploring how these genes can promote the progression of AMD by affecting autophagy, cellular senescence, and inflammatory responses, to provide definite molecular mechanisms for future research and lay a foundation for precision medicine as well as drug development for AMD. Ultimately, through the drug-gene interactions, we identified target drugs of the hub genes, which could be available for AMD treatment, putting forward an idea that if the application of these drugs for targeting different hub genes can be combined, it is possible to achieve the effects of anti-neovascularization, improving autophagy function, alleviating cellular senescence, and inhibiting the inflammatory response, based on the activation or inhibition interactions of the five hub genes, providing a new insight into AMD treatment.

# Methods

#### Text Mining

Text mining was performed by pubmed2ensembl (http://pubmed2ensembl.ls.manchester.ac.uk/), which reveals links between genes and the literature for data excavation. Briefly, after queries are executed, all genes related to the concepts were extracted by pubmed2ensembl from available biological literature.<sup>21,22</sup> In this study, "Homo sapiens" was set as the species. Four queries were performed using the search terms "age-related macular degeneration," "autophagy," "cellular senescence," and "inflammatory response," and then "search for PubMed IDs," "retrieve up to 100000 document IDs," and "filter on MEDLINE PubMed ID" were chosen to produce a list of genes. We deleted the duplicates from each gene set and obtained the intersection of these four gene sets. which was then used in the subsequent analyses.

### GO Biological Process and KEGG Pathway Analyses

GeneCodis (http://genecodis.cnb.csic.es), which interprets gene lists via enrichment analysis and integration of various biological information, 23-25 was used for the enrichment analysis of the gene intersection of the previous step. The genes from the intersection were entered into the input field and analyzed by the GO biological process annotations. "Homo sapiens" was set as the organism. Then, we chose "Hypergeometric" as the statistical test and "FDR" [false discovery rate] to correct the P value. The *P* value cut-off was set up to select the most enriched biological processes related to AMD. The genes from the selected annotations were used for the KEGG pathway analysis using the same method, and the genes obtained by the KEGG pathway analysis were further analyzed.

# **Protein–Protein Interactions**

The STRING database (http://string-db.org) was used to analyze the PPI network of the selected genes. "Multiple proteins" and "*Homo sapiens*" were selected. Higher confidence scores indicate a stronger interaction between the two proteins, whereas lower confidence scores indicate the use of more broadened inclusion criteria. "Evidence," which indicates the type of interaction by line color, or "molecular action," which indicates the predicted mode of action by line



**Figure 1.** An overview of the strategy in this study. Text mining was used to identify genes associated with the concepts "age-related macular degeneration," "autophagy," "cellular senescence," and "inflammatory response" using pubmed2ensembl. The selected genes were used for functional analysis using the GeneCodis. Further enrichment was obtained by protein–protein interaction analysis using STRING and Cytoscape. The final drug list was obtained by drug–gene interaction analysis using the DGldb.

shape, was set as the meaning of network edges. The file of ".tsv" format was downloaded for the subsequent step.

Next, the Cytoscape software, which visually presents the integration of gene expression, biological network, and genotype,<sup>26</sup> was used to visualize the interaction network and determine hub genes. CentiScaPe, an APP that predicts the network using graphical and numerical output,<sup>27</sup> was then used to determine key nodes. "Degree" and "Betweenness" were chosen as parameters. In this study, we selected the nodes with degree and betweenness values greater than or equal to the average as hub genes for the analysis in the next step.

#### **Drug–Gene Interactions**

The DGIdb database version 3.0 (http://www. dgidb.org), which includes drug–gene interaction data from 30 different sources,<sup>28</sup> was used to explore the potential targets of existing associations with drugs or



#### Table 1. Results of Text Mining

CD34, IL10, REN, SOAT1, FASLG, PHGDH, TNFSF10, CXCR4, LPA, ESR1, IL1B, NOS3, ATM, LEP, BAX, MMP1, CAV1, TGFB1, FADD, VEGFA, FAS, CDC42, TLR4, MAPK14, HGF, BRCA1, IFNG, TLR3, TNFRSF1B, MMP2, IFNA1, AHR, HMOX1, BDNF, IL6, FOS, CYCS, CAT, NFKB1, IL4, PPARG, NOS2, IL2, CD14, MAPK3, TNF, CCL2, EDA, MAPK8, MMP9, CD40, AKT1, CXCL8, HIF1A, TP53, MAPK1, PRKAA1, BCL2, HMGB1, CD4, APP, APEX1

These 62 genes were common to the four queries "age-related macular degeneration," "autophagy," "cellular senescence," and "inflammatory response."

small organic compounds of the final list of genes. At least one drug or small organic compound was discovered to correspond with one gene. We entered the hub genes from Cytoscape into the input set and selected "FDA-Approved" (U.S. Food and Drug Administration) as the filter. Finally, targeted drugs with therapeutic potential in AMD were sorted out. For more details from the output of the databases or softwares, please see the Supplementary Material Visual Method.

## **Results**

#### **Results of Text Mining**

The research strategy of our work is summarized in Figures 1 and 2. The number of genes related to AMD, autophagy, cellular senescence, and the inflammatory response were 719, 671, 1505, and 423, respectively, and 62 genes were common to the four lists (Table 1).

# Results of GO Biological Process and KEGG Pathway Analyses

The 62 genes were analyzed for GO biological processes using GeneCodis, which revealed the highly enriched terms that were strongly associated with AMD pathology. To ensure only the most enriched annotations relevant to AMD pathology were selected, a corrected P value cut-off (P = 1.00E-11) was set. If a *P* value (P = 1.00E-12) was set as the cut-off, "Aging," a biological processes that contributes significantly to AMD pathology, 29-31 would be excluded. The relatively low P value and higher gene representation made the process particularly relevant to AMD. We found that there were several situations in annotations with a *P* value of greater than or equal to 1.00E-10: (1) The annotations related to AMD pathophysiologes that had been selected previously would reappear, and they would not be chosen again; (2) The annotations irrelevant to AMD pathophysiologes, whether they were new or repeated, would not be selected

owing to their irrelevance; and (3) There were new annotations related to AMD pathophysiologes with larger P values and less enriched genes. Nevertheless, the change of P value from "Response to Hypoxia (P = 4.05593E-25)" to "Aging (P = 1.3252E-12)" has gone through 13 orders of magnitude, and P values of greater than or equal to 1.00E-10 are already small compared with P = 4.05593E-25 of "Response to hypoxia," indicating the correlations of these annotations with AMD are relatively weak. To narrow the scope to screen genes, the annotations with a P value of greater than or equal to 1.00E-10 were not considered. Among the most significantly enriched annotations below the cut-off, we identified 19 biological processes that most related to AMD pathology according to the available literature and research, in which 42 unique genes were contained (Table 2). The five most enriched biological process annotations were (1) "Response to hypoxia" (P = 4.05593E-25); (2) "Anti-apoptosis" (P = 2.12E-17); (3) "Lipopolysaccharide-mediated signaling pathway" (P = 3.06093E-17); (4) "Signal transduction" (P = 4.79281E-16); and (5) "Positive regulation of nitric oxide biosynthetic process" (P = 2.53E-15), containing 18, 14, 9, 9, and 6 genes, respectively. Other enriched biological processes included "Inflammatory response," "Platelet activation," "Negative regulation of cell proliferation," "Blood coagulation," "Activation of MAPK activity," "Positive regulation of chemokine biosynthetic process," "Innate immune response," "Response to mechanical stimulus," "Response to glucocorticoid stimulus," "Positive regulation of apoptotic process," "Positive regulation of NF [nuclear factor]-kappaB transcription factor activity," "Humoral immune response," "Positive regulation of angiogenesis," and "Aging."

Next, the 42 genes were analyzed for KEGG pathways using GeneCodis. To obtain the most enriched annotations, a corrected P value cut-off (P = 1.00E-10) was set. If a P value (P = 1.00E-11) was set as the cut-off, "VEGF signaling pathway," which contributes significantly to AMD pathology, would be excluded. The reasons why annotations with a P value

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Table 2.

Process	Genes in Query Set	Total Genes in Genome	FDR-Corrected Hypergeometric <i>P</i> Value <sup>*</sup>	Genes
Response to hypoxia	18	175	4.05593E-25	VEGFA, TNF, BCL2, CAV1, CCL2, HIF1A, HMOX1, FAS, LEP, TLR4, MMP2, NOS2, TGFB1, NOS3, CAT, ATM, IL1B, MMP9
Antiapoptosis	14	200	2.12E-17	NFKB1, VEGFA, TNF, BCL2, CCL2, HMOX1, FAS, BDNF, AKT1, IL10, HGF, IL2, NOS3, IL1B
Lipopolysaccharide-mediated signaling pathway	6	29	3.06093E-17	MAPK3, MAPK1, TNF, CCL2, TLR4, MAPK14, TGFB1, NOS3, IL1B
Signal transduction	6	39	4.79281E-16	TNF, CCL2, HIF1A, HMOX1, FAS, TLR4, NOS3, ATM, IL1B
Positive regulation of nitric oxide biosynthetic process	9	7	2.5284E-15	IFNG, TNF, TLR4, AKT1, IL6, IL1B
Inflammatory response	80	31	5.57302E-15	FOS, NFKB1, TNF, AKT1, IL10, TGFB1, IL6, IL18
Platelet activation	7	17	7.66934E-15	MAPK3, VEGFA, APP, AKT1, MAPK14, TGFB1, IL6
Negative regulation of cell proliferation	80	35	1.27788E-14	PPARG, TNF, CAV1, TP53, TGFB1, NOS3, IL6, IL1B
Blood coagulation	15	457	1.63007E-14	CDC42, MAPK3, VEGFA, MAPK1, IFNA1, CAV1, APP, TP53, AKT1, NOS2, HGF, MMP1, MAPK14, TGFB1, NOS3
Activation of MAPK activity	9	6	1.69449E-14	MAPK3, MAPK1, TNF, TLR4, MAPK14, IL1B
Positive regulation of chemokine biosynthetic process	9	10	3.11065E-14	IFNG, TNF, HMOX1, IL4, TLR3, IL1B
Innate immune response	13	309	4.57179E-14	FOS, NFKB1, PPARG, MAPK3, MAPK1, IFNA1, BCL2, APF, TLR4, MAPK8, MAPK14, CD14, HMGB1
Response to mechanical stimulus	7	23	5.18346E-14	FOS, TNF, CAV1, CCL2, NOS3, IL6, MMP9
Response to glucocorticoid stimulus	7	27	1.47011E-13	TNF, CAV1, CCL2, FAS, IL10, IL6, IL1B
Positive regulation of apoptotic process	9	13	1.79318E-13	FAS, TLR4, NOS3, ATM, IL1B, MMP9
Positive regulation of NF-kappaB transcription factor activity	6	92	2.81499E-13	NFKB1, TNF, EDA, TLR4, TLR3, TGFB1, CAT, IL6, IL1B
Humoral immune response	Ŋ	9	3.31024E-13	IFNG, TNF, BCL2, CCL2, IL6
Positive regulation of angiogenesis	9	16	6.78039E-13	VEGFA, HIF1A, HMOX1, NOS3, IL1B, MMP9
Aging	7	38	1.3252E-12	FOS, CCL2, FAS, TGFB1, NOS3, IL6, IL1B

To obtain the most enriched annotations, a corrected P value cut-off (P = 1.00E-11) was set. Among the most significantly enriched annotations below the cut-off, we identified 19 biological processes that most related to AMD pathology according to the available literature and research, in which 42 unique genes were contained.

\*FDR stands for false discovery rate. The FDR correction was performed to control for the false positives expected with a large number of comparisons.

of greater than or equal to 1.00E-9 were not considered are the same as those of GO biological process analysis for excluding annotations with P values of greater than or equal to 1.00E-10. Among the most significantly annotations below the cut-off, we identified 11 pathways that most related to AMD pathology based on the available literature and studies, in which 37 unique genes were contained (Table 3). The five most enriched pathway annotations were (1) "Pathways in cancer" (P = 1.43089E-27); (2) "Toll-like receptor signaling pathway" (P = 4.97021E-22); (3) "Cytokinecytokine receptor interaction" (P = 8.65209E-19); (4) "MAPK signaling pathway" (P = 9.80753E-19); and (5) "T-cell receptor signaling pathway" (P = 6.70871E-18); each containing 20, 13, 14, 14, and 11 genes, respectively. Other enriched pathways included "NOD-like receptor signaling pathway," "Neurotrophin signaling pathway," "Focal adhesion," "VEGF signaling pathway," "Apoptosis," and "Jak-STAT signaling pathway."

#### **Results of Protein–Protein Interactions**

The PPI networks of the 37 genes was illustrated using STRING (Fig. 3). To screen the genes with a strong interaction and to further narrow the candidate gene field, "Evidence," and "highest confidence (0.9)" were selected. Then, we used CentiScaPe of the Cytoscape software to select the key nodes of the PPI network (Fig. 4). Nodes in a network can be genes, proteins, or molecules, and connections represent the interactions between the nodes.<sup>26</sup> "Degree" and "Betweenness" were selected as parameters on behalf of centralities. In this study, the minimum and maximum degree values were 1 and 15, respectively, with an average value of 6.6285714, and the minimum and maximum betweenness values were 0 and 220.8126596, respectively, with an average value of 40.9142857. The degree represents the total number of edges connected to a node, and the betweenness is the ratio of the number of shortest paths passing through a node to the total number of paths passing through the node. A higher degree of a node indicates that more genes interact with that node, and a greater betweenness value of a node suggests a greater influence of this gene on the regulation of the whole network. Nodes with higher degrees and greater betweenness values tend to be more necessary to the whole network.<sup>26</sup> We selected key nodes with both degree and betweenness values higher than or equal to the mean and obtained the following 12 genes (Table 4): IL-6, VEGF-A, TP53, AKT1, NF $\kappa$ B1, MAPK8, IL-2, MAPK3, MAPK1, MAPK14, IL-1 $\beta$ , and transforming growth factor (TGF)- $\beta$ 1.

AKT1, MAPK8, MAPK3, MAPK1, and MAPK14 are involved in the regulation of the MAPK pathway, which was one of the five most enriched pathway annotations confirmed by KEGG pathway analysis in this study (Table 3). However, the MAPK pathway is a complex cascade composed of many proteins, and MAPK kinases act as mediators in multiple signaling pathways, activating and phosphorylating downstream steps after activation,<sup>32</sup> and the PPI network demonstrated the central role of AKT1, MAPK8, MAPK3, MAPK1, and MAPK14 (Fig. 4). NF $\kappa$ B1 encodes the p50 homodimer, which inhibits NF- $\kappa$ B and is involved in regulating aging and the inflammatory response.  $NF\kappa B1^{-/-}$  mice display enhanced inflammation and are susceptible to DNA damage, resulting in a rapid aging phenotype.<sup>33–35</sup> Hence. NF $\kappa$ B1 acts as an inhibitor of aging and the inflammatory response. However, Alu RNA, a pathogenic factor in geographic atrophy in dry AMD, could induce RPE cell degeneration by activating NF $\kappa$ B, while NF $\kappa$ B1<sup>-/-</sup> mice were protected against Alu RNA-induced degeneration of RPE cells,<sup>36</sup> indicating that  $NF\kappa B1$  is a risk factor for AMD, which conflicts with the results showing that  $NF\kappa B1$ inhibits aging and the inflammatory response. IL-2, which is mainly generated by activated T lymphocytes, is involved in the T-cell receptor signaling pathway,<sup>37</sup> corresponding with the results of KEGG pathway analysis (Table 3). IL-2 is a proinflammatory factor.<sup>47</sup> The incubation of RPE cells with A2E, a central spontaneous fluorophore of lipofuscin implicated in AMD, increases inflammatory factors, including IL-1 $\beta$ , ICAM, IL-6, IL-2, and IL-8.<sup>48</sup> IL-2 also induces autophagy-related cell death.<sup>38</sup>

Therefore, considering the role of NF $\kappa$ B1 in cellular senescence and the inflammatory response in AMD requires further in-depth study and that targeting IL-2 may not be effective in suppressing inflammation or increasing autophagy, and considering that the application of drugs on MAPK pathway-related genes may affect multiple biological processes in addition to autophagy, cellular senescence, and the inflammatory response with unpredictable results, we finally selected IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1 as hub genes. To further demonstrate that these five hub genes are from RPE cells, we used "retina pigment epithelium" as a search concept in pubmed2ensembl, and obtained 753 genes. Finally, 53 genes were common to the five concepts "age-related macular degeneration," "retina pigment epithelium," "autophagy," "cellular senescence," and "inflammatory response" (Supplementary Table S1). It can be seen that IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1 were included in genes from the RPE, indicating that these five key genes are indeed related to RPE cells. The PPI network of the five

Process	Genes in Query Set	Total Genes in Genome	Hypergeometric P Value*	Genes
Pathways in cancer	20	324	1.43E-27	IL6, MMP9, CDC42, MAPK1, MAPK3, FOS, HIF1A, MMP2, FAS, MAPK8, AKT1, NFKB1, TGFB1, NOS2, BCL2, TP53, HGF, VEGFA, PPARG, MMP1
TLR signaling pathway	13	101	4.97E-22	ILIB, ILG, MAPK1, MAPK3, FOS, TLR3, IFNA1, TLR4, MAPK8, AKT1, NFKB1, CD14, MAPK14
Cytokine-cytokine receptor interaction	14	259	8.65E-19	IL18, IL6, IL2, FAS, IFNG, EDA, IFNA1, IL10, CCL2, TGFB1, LE7, IL4, HGF, VEGFA
MAPK signaling pathway	14	262	9.81E-19	IL1B, CDC42, MAPK1, MAPK3, FOS, BDNF, FAS, MAPK8, AKT1, NFKB1, TGFB1, CD14, MAPK14, TP53
T-cell receptor signaling pathway	11	107	6.71E-18	CDC42, MAPK1, MAPK3, FOS, IL2, IFNG, IL10, AKT1, NFKB1, MAPK14, IL4
NOD-like receptor signaling pathway	8	22	8.02E-18	ILIB, ILG, MAPK1, MAPK3, MAPK8, NFKB1, CCL2, MAPK14
Neurotrophin signaling pathway	10	124	2.03E-15	CDC42, MAPK1, MAPK3, BDNF, MAPK8, AKT1, NFKB1, MAPK14, BCL2, TP53
Focal adhesion	6	197	5.06E-12	CDC42, MAPK1, MAPK3, CAV1, MAPK8, AKT1, BCL2, HGF, VEGFA
VEGF signaling pathway	7	75	1.04E-11	CDC42, MAPK1, MAPK3, AKT1, NOS3, MAPK14, VEGFA
Apoptosis	7	86	2.60E-11	ILIB, FAS, AKT1, NFKB1, BCL2, TP53, ATM
Jak–STAT signaling pathway	8	153	2.86E-11	ILG, ILZ, IFNG, IFNA1, IL10, AKT1, LEP, IL4
To obtain the most enrich	ed annot	ations, a col	rrected <i>P</i> value	cut-off ( $P = 1.00E-10$ ) was set. Among the most significantly annotations below the cut-off, we

# Summary of KEGG Pathway Analysis Table 3.

FDR-Corrected

identified 11 pathways that most related to AMD pathology based on the available literature and studies, in which 37 unique genes were contained. \*FDR stands for false discovery rate. The FDR correction was performed to control for the false positives expected with a large number of comparisons.



Figure 3. High confidence PPI network of the 37 enriched genes from KEGG pathway analysis by STRING. Connecting line color indicates the types of interaction evidence with the confidence score set at 90%.



**Figure 4.** The PPI network of the 37 enriched genes from KEGG pathway analysis by Cytoscape. (**a**) "Degree" was set to represent the centrality of the PPI network. Purple nodes represent that their degree values were higher than or equal to the mean. The darker the color, the higher the value. (**b**) "Betweenness" was set to represent the centrality of the PPI network. Red nodes represent that their betweenness values were higher than or equal to the mean. The darker the color, the higher the value.



Figure 5. Medium confidence of the PPI network of the 5 hub genes by STRING. Connecting line shapes indicate the predicted mode of action with the confidence score set at 40%.

Table 4.	Selection	of Hub	Genes	by C	ytoscape
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Number	Gene	Degree Value	Betweenness Value
1	IL6	15	220.8126596
2	VEGFA	11	162.3981407
3	TP53	13	152.7138528
4	AKT1	13	152.6429737
5	NFKB1	9	76.95981241
6	MAPK8	12	74.05841381
7	IL2	10	66.36393051
8	MAPK3	13	58.91906427
9	MAPK1	12	53.72541348
10	MAPK14	10	48.08202353
11	IL1B	10	44.3033633
12	TGFB1	8	44.2959596

The average degree and betweenness values were 6.6285714 and 40.9142857, respectively. Twelve genes were selected with both degree and betweenness greater than or equal to the mean.

genes was diagrammatized using STRING. "Molecular action" and "medium confidence (0.4)" were selected to broaden the inclusion criteria and identify the interaction modes (activation/inhibition/unknown) between the five genes (Fig. 5). VEGF-A could be activated or inhibited by IL-6, IL-1 $\beta$ , TP53, and TGF- $\beta$ 1, and IL-6 could be activated or inhibited by IL-1 $\beta$ , TP53, and TGF- $\beta$ 1. The activation or inhibition effect between IL-1 $\beta$ , TP53, TGF- $\beta$ 1, and other nodes was weaker than that of IL-6 and VEGF-A. If applying the number of network edges to indicate the strength of the activation or inhibition role between nodes, VEGF-A, IL-6, IL-1 $\beta$ , TP53, and TGF- $\beta$ 1 had 6, 6, 3, 3, and 2, respectively.

#### **Results of Drug–Gene Interactions**

The drug-gene interactions of IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1 were analyzed using the DGIdb, and 133 FDA-approved drugs were identified (data not shown). After consulting the literature, we summarized and identified 24 target drugs with therapeutic potential for AMD (Table 5). Among these, 11 drugs have been used in the treatment of AMD, and 10 drugs have been shown to be effective.

## Discussion

Autophagy dysfunction and cellular senescence of RPE cells and the retinal inflammatory response are the main pathologic factors in AMD, which has been reviewed in our previously work in 2019.<sup>9</sup> If improvements in autophagy, the alleviation of cellular senescence, and the inhibition of abnormal inflammatory responses were achieved comprehensively, it would be possible to delay the progression of AMD. In this study, we identified 62 genes collectively involved in

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Numb	er Drug	Gene	Drug–Gene Interaction	Score*	Approved?	AMD?	Approved Use	References (Pubmed ID)	for AMD
-	Saquinavir	IL6	I	2	Yes	No	AIH	15388451	I
2	Nelfinavir	IL6	I	2	Yes	No	HIV	15388451	ı
e	Fenofibrate	VEGFA	I	2	Yes	No	Hyperlipidemia	11356390	ı
4	Bevacizumab	VEGFA	Antibody inhibitor	10	Yes	Yes	Neovascular AMD	27079204	Intravitreal injection
5	Gliclazide	VEGFA	. 1	5	Yes	No	Retinal neovascularization	17602961	I
9	Sorafenib tosylate	VEGFA	Inhibitor	-	Yes	Yes	Neovascular AMD	18241635	Oral
7	Carvedilol	VEGFA	I	5	Yes	Yes	AMD, heart failure	19258246, 15732037	I
8	Pegaptanib sodium	VEGFA	Antagonist	-	Yes	Yes	Neovascular AMD	25733493	Intravitreal injection
6	Ranibizumab	VEGFA	Inhibitor	14	Yes	Yes	Neovascular AMD	26996339, 15973626	Intravitreal injection
10	Vandetanib	VEGFA	Inhibitor	-	Yes	No	Retina neovascular disease	28413487	I
11	Aflibercept	VEGFA	Binder  Antibody  Inhibitor	7	Yes	Yes	Neovascular AMD	23766432, 22813448	Intravitreal injection
12	Enalapril	TP53	I	2	Yes	No	Ventricular hypertrophy	16900775	I
13	Zinc chloride	TP53	I	2	Yes	No	Prostate dysplasias	16585387	I
14	Methylprednisolone	TP53	I	2	Yes	Yes	AMD, psoriasis	17469755, 16684279	Parabulbar injection
15	Bortezomib	TP53	Inhibitor	-	Yes	No	I	I	I
16	Canakinumab	IL1B	Binder  Antibody  Inhibitor	7	Yes	No	Inflammatory disorders	19169963	I
17	Raloxifene	IL1B	I	2	Yes	No	I	12773123	I
18	Rilonacept	IL1B	Binder  Inhibitor	5	Yes	No	Acute gouty arthritis	23319019, 23553601	I
19	Pioglitazone	TGFB1	1	2	Yes	Yes	AMD, liver fibrosis	19258246, 17407709	I
20	Triamcinolone	TGFB1	I	2	Yes	Yes	Neovascular AMD, keloid	20640976, 12174062	Intravitreal injection
21	Glatiramer acetate	TGFB1	I	-	Yes	Yes	Dry AMD	21254921	Subcutaneous injection
22	Ramipril	TGFB1	I	2	Yes	No	Hypertension	15716710	I
23	Amifostine	TGFB1	I	2	Yes	No	Radiation-induced pulmonary toxicity	1 2005 544	I
24	Vitamin E	TGFB1	I	e	Yes	Yes	AMD (noneffective), liver fibrosis, IgA nephropathy	28756617, 1505665, 9608545	I
╞╾┾	venty-four drugs v he crore is the con	vith thera	apeutic potential in AN	ID were	e identifie d PubMé	d in the	e final list. ences sumorting a given interaction		
-			מווחרו כו ממימאמיר יכי				רוורריז זמשלאסו נוווא מ אוייריו וווירומריוטווי		

 Table 5.
 Candidate Drugs Targeting Genes for AMD

translational vision science & technology-

#### Genes and Drug Discovery in AMD

FDA, U.S. Food and Drug Administration.

AMD, autophagy, cellular senescence, and the inflammatory response (Table 1) via text mining, 19 biological processes including 42 genes (Table 2) via GO biological process analysis, 11 enriched KEGG pathways including 37 genes (Table 3) via KEGG pathway analysis, and 12 hub genes (Fig. 3, Fig. 4, and Table 4) via PPI analysis step by step. Finally, five hub genes (IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1) and their specific interaction modes were identified (Fig. 5), corresponding with 24 target drugs with therapeutic potential for AMD (Table 5). This study provided definite molecular mechanisms for further research and new possibilities for AMD treatment in the future.

The results of GO biological process revealed the most enriched annotations which are closely related to the pathology of AMD. For example, the most enriched biological process annotations were "Response to hypoxia," "Anti-apoptosis," and "Lipopolysaccharide-mediated signaling pathway." and so on (Table 2). The retina is a tissue that is rich in vessels and has high metabolic requirements. It is known that the macula receives the highest blood flow of any tissue in the body when relative to its size.<sup>39</sup> Hypoxia-inducible factors (HIFs) are key molecules of hypoxia response. Under physiologic conditions, HIFs are not required.<sup>40</sup> During tissue development, vascular networks are constructed using hypoxia response.<sup>41</sup> Pathologic HIF activation is related to the abnormalities the of retinal vasculature and hemorrhage.<sup>42</sup> Long-term hypoxia can mediate VEGF production, a key regulator in neovascularization, and lead to chronic retinal ischemia and eventually irreversible vision loss.<sup>39</sup> In addition to the role in angiogenesis. HIFs are also a regulator of autophagy. and hypoxia can be a stimuli for inflammation.<sup>4</sup> Moreover, under high metabolic conditions, such as inflammation, the metabolic activity and oxygen consumption of the inflamed retinal cells increase, resulting in hypoxia. Inflammation and local hypoxia can be present in aging choriocapillaris, RPE cells, and the neural retina.<sup>39,43</sup> Thus, it can be seen that hypoxia is closely related to the pathology of AMD, but it is not limited to these processes, indicating that the result of our analysis is consistent with the existing knowledge. Apoptosis is involved in the AMD-related death of RPE cells.<sup>44</sup> Autophagy can antagonize apoptosis-induced cell death by removing damaged organelles, and cells may fail to survive under inhibited autophagy.<sup>45</sup> DNA damage is one of the hallmarks of cellular senescence, and apoptosis can be induced by serious DNA damage.<sup>46</sup> One of the characteristics of senescent cells is apoptosis resistance.<sup>47</sup> It was reported that lipopolysaccharide can induce an inflammatory response of RPE cells with the activation of

IL-1 $\beta$ , IL-6, IL-8, phospholipases D 1 and 2 and cyclo-oxygenase-2.<sup>48,49</sup>

The most highly enriched pathway annotations were "Pathways in cancer," "Toll-like receptor signaling pathway," and "Cytokine-cytokine receptor interaction" (Table 3), which are relevant to the pathology of AMD. A tumor is a complex disease involving multiple biological processes and cytokines. By consulting the KEGG pathway database (https:// www.genome.jp/kegg/pathway.html), we found that the "Pathways in cancer" is very complicated and broad, which contains "Cytokine-cytokine receptor interaction," "MAPK signaling pathway," and "VEGF signaling pathway," and so on. Hence, we thought that most genes that participated in AMD are involved in this pathway, so that the most enriched KEGG pathway annotation was "Pathways in cancer." As is well-known, innate and adaptive immune effectors can be regulated in the eve. and RPE cells can contribute to the immune-privileged property of the eve. The Toll-like receptor (TLR) signaling pathway is involved in the innate immune response.<sup>50</sup> Studies showed that TLR3 protected RPE cells from oxidative stress through a STAT3-dependent mechanism,<sup>51</sup> whereas TLR2 played a role in facilitating oxidative stressinduced retinal degeneration.<sup>50</sup> Many cytokines are involved in the pathway "Cytokine-cytokine receptor interaction," including the chemokines, TNF family, TGF- $\beta$  family, and interleukin family, which extensively participate in autophagy, cellular senescence, and inflammatory response. 47, 52, 53

IL-6 and IL-1 $\hat{\beta}$  are major markers of the inflammatory response.<sup>54</sup> They also promote the aging process as senescence-associated secretory phenotype (SASP) components<sup>55</sup> and participate in "Toll-like receptor signaling pathway," and "Cytokine-cytokine receptor interaction,"56,57 corresponding with the results of the KEGG pathway analysis (Table 3). Although senescent cells cannot proliferate, they still have active metabolic capacity for a long time, accompanied by the secretion of SASP, which promotes a series of inflammatory cascades and accelerate cellular senescence.<sup>9</sup> The relationship of IL-6 and IL-1 $\beta$  involvement in autophagy, cellular senescence, and the inflammatory response is shown in Figure 6. Studies have shown that autophagy decreases in aged mice<sup>58,59</sup> and that the proinflammatory factors IL-6 and TNF- $\alpha$  increase.<sup>59</sup> TNF- $\alpha$  in turn inhibits autophagy.<sup>60</sup> IL-1 $\beta$  expression is increased in senescent RPE cells.<sup>61</sup> Many endogenous damage-associated molecular patterns can accumulate during aging, and age-related damage-associated molecular patterns activate the NLRP3 inflammasome, leading to IL-1 $\beta$  release. This process in turn results in pathologic low-grade inflammation and further IL-

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Figure 6. The mechanism by which IL-6 and IL-1 $\beta$  is involved in autophagy, cellular senescence, and the inflammatory response.

6 release.<sup>62</sup> Impaired autophagy, on the one hand, promotes the release of IL-6<sup>58,63</sup> and, on the other hand, prevents the degradation of cytoplasmic NF- $\kappa$ B p65 and stabilizes nuclear NF- $\kappa$ B p65, which further continuously activates IL-1 $\beta$  and amplifies the inflammatory response.<sup>64</sup> Impaired autophagy also leads to cellular senescence<sup>65</sup> and a shortened lifetime.<sup>62</sup> The activated inflammatory response promotes NF- $\kappa$ B activation and IL-6 release, which further mediates cellular senescence. The senescent cells upregulate IL-6 expression by secreting SASP.<sup>65</sup>

The mechanism of TP53 (p53) involvement in cellular senescence, autophagy, and the inflammatory response is shown in Figure 7. Mechanistically, cellular senescence relies on two main molecular pathways: the p16<sup>INK4A</sup>–pRB and p53–p21<sup>CIP1/WAF1</sup> pathways. Upon activation, p53 upregulates p21<sup>CIP1/WAF1</sup>, which inhibits the cell cycle proteins cyclin A, E, and D.<sup>66,67</sup> On the one hand, senescent cells promote inflammation by secreting SASP to release inflammation-related cytokines. On the other hand, senescent cells secrete HMGB1 to activate the TLR-4 signaling pathway and NF- $\kappa$ B, further mediating IL-6 release.<sup>68</sup> p53 can either repress or activate autophagy relying on its subcellular location. Nuclear p53 induces autophagy

through the upregulation of AMPK and PTEN while cytoplasmic p53 represses autophagic flux by inhibiting AMPK and activating mammalian target of rapamycin.<sup>66,69</sup> Autophagy activation can downregulate AMPK and suppress oxidative stress by eliminating reactive oxygen species (ROS), thereby limiting p53 activity. In addition, p53 is an important marker of DNA damage. Autophagy may limit p53 activation by providing autophagic substrates that contain many antioxidant components that are beneficial for DNA replication and repair, thereby preventing DNA damage.<sup>70</sup> p53 also plays a dual role in regulating the inflammatory response. It can inhibit the inflammatory response by suppressing NF- $\kappa$ B,<sup>71</sup> and it can also induce the expression of proinflammatory cytokines by mediating the activation of TLRs or cooperating with  $NF-\kappa B.^{72}$ 

In addition to being involved in autophagy, cellular senescence and the inflammatory response, TGF- $\beta$ 1 is also a major factor in epithelial mesenchymal transformation (EMT),<sup>73</sup> a process through which epithelial cells lose their epithelial characteristics.<sup>74</sup> RPE cells easily undergo EMT and that causes changes in the cellular morphology and functions, which is one of the identified risk factors for AMD. Exposure





Figure 7. The mechanism by which TP53 is involved in autophagy, cellular senescence, and the inflammatory response.

the secretion of TGF- $\beta$ 1 and upregulation of EMT markers.<sup>75,76</sup> The mechanism of TGF- $\beta$ 1 involvement in cellular senescence, autophagy and the inflammatory response is summarized in Figure 8. Briefly, TGF- $\beta$ 1 induces cellular senescence by (1) upregulating NOX4 through SMAD2/3, resulting in increased intracellular ROS levels and  $H_2O_2$  release<sup>77,78</sup>; (2) activating the p38 MAPK pathway<sup>79</sup>; and (3) inhibiting GSK3 activation by phosphorylating PKCδ, resulting in mitochondrial dysfunction and elevated ROS.<sup>80</sup> TGF- $\beta$ 1 is one of the SASP components<sup>79</sup>; therefore, senescent cells can promote TGF- $\beta$ 1 expression by secreting SASP. In terms of autophagy, TGF- $\beta$ 1 has promoting as well as inhibitory effects. TGF- $\beta$ 1 can promote autophagy by accelerating ROS release or activating the TAK1-induced MKK3-p38 pathway,<sup>81</sup>

whereas it down-regulates the level of autophagy by activating the PI3K-AKT-mamallian target of rapamycin and MAPK-ERK1/2 pathways.<sup>82</sup> TGF- $\beta$ 1 is considered as a double-edged sword in the inflammatory response. TGF- $\beta$ 1 can inhibit inflammatory responses by<sup>83</sup> (1) activating SMAD-dependent pathways; (2) activating SMAD-independent (ERK, p38, JNK, etc.) pathways, further promoting EMT; and (3) activating immune cells such as Th17, Th9, and regulatory T cells. In contrast, TGF- $\beta$ 1 alone can induce the expression of the inflammatory cytokine CCL2. When cooperating with TNF- $\alpha$ , TGF- $\beta$ 1 upregulates CCL2, IL-8, and cyclooxygenase-2 through NF- $\kappa$  B p65, SMAD3, and TAK1, respectively.<sup>84</sup>

It has been demonstrated that VEGF plays a key role in both angiogenesis and vascular Genes and Drug Discovery in AMD



Figure 8. The mechanism by which TGF- $\beta$ 1 is involved in autophagy, cellular senescence, the inflammatory response, and AMD.

permeability,<sup>85</sup> which is known as an important risk factor in the occurrence and development of wet AMD.<sup>86</sup> The mechanism of VEGF-A involvement in cellular senescence, autophagy, and the inflammatory response is summarized in Figure 9. The upregulation of VEGF-A is mediated mainly by hypoxia,<sup>86</sup> corresponding with the GO analysis results, which showed that the most enriched biological process annotation was "Response to hypoxia" (Table 2). Other factors include oxidative stress and high glucose. These three factors promote VEGF-A expression by inducing HIF-1 $\alpha$ , IL-6, and AGE, respectively.<sup>86</sup> In AMD, autophagy dysfunction in RPE cells promotes a low-grade inflammatory response, leading to IL-1 $\beta$  release. In addition, impaired autophagy in RPE cells is often accompanied by mitochondrial dysfunction, and then ROS is increased, which leads to the upregulation of IL-6 and IL-1 $\beta$  through NLRP3 and TNF- $\alpha$ . IL-6 and IL-1 $\beta$  further upregulate VEGF-A and result in RPE cell degeneration.<sup>86–88</sup> Moreover,



Figure 9. The mechanism by which VEGF-A is involved in autophagy, cellular senescence, the inflammatory response, and AMD.

as a SASP component, VEGF-A can activate ERK, promote oxidative stress damage, and induce RPE cellular senescence via NLRP3-mediated IL-1 $\beta$  expression.<sup>88–90</sup>

In summary, IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1 are pivotal in autophagy, cellular senescence, and the inflammatory response in AMD. Targeting these five genes is expected to increase autophagy, alleviate cellular senescence, and inhibit the inflammatory response. The specific interaction of the five genes is clearly shown in Figure 5. When applying drugs to target genes, the effect of activation or inhibition of one gene on the other four genes should be taken into account to achieve better clinical effects.

The application of bioinformatics offers powerful tools that reveal the molecular mechanisms of diseases. Text mining provides a wealth of genes associated with diseases. GO biological process and KEGG pathway analyses can gradually screen out the enriched genes associated with the diseases, by which the unique genes obtained from text mining can be presented in an integrated form through functional annotations. The PPI visualization and analysis of enriched genes via STRING and Cytoscape presents perceivable framework and identifies key nodes that have significant influence on the network. The analysis of drug-gene interactions via DGIdb identifies potential drugs that target key genes. Because this study identified the potential genes and target drugs that are collectively involved in the three biological processes in AMD though a series of systematic bioinformatic methods, there are some limitations in the present study. (1) Text mining has not revealed information on the contextual environment and positive and negative relations. GO biological process and KEGG pathway analyses reflect the functions of genes relying on annotations, which may overemphasize highly conserved cellular processes and may neglect some tissue-specific functions,

unnecessarily limiting what can be seen. In addition, because the databases used in this study are constantly updated, the genes or pathways obtained from the databases may be limited. Hence, more and better methods need to be applied in the future and our analysis work needs to be repeated to obtain more accurate information. (2) The criteria for setting P values are subjective, and so subsequent studies can set different criteria to further explore the best results. (3) Finally, further experiments are still needed for confirming the roles of the key genes and the effect of the target drugs.

# Conclusions

Based on text mining, we used GO biological process, KEGG pathway, and PPI analyses to identify that IL-6, VEGF-A, TP53, IL-1 $\beta$ , and TGF- $\beta$ 1 are pivotal in autophagy, cellular senescence and the inflammatory response in AMD and define their specific interaction modes with each other, providing definite molecular mechanisms for future research and laying a foundation for precision medicine as well as drug development for AMD. On this basis, we identified 24 drugs with therapeutic potential in AMD, providing new possibilities for AMD treatment. For the 10 effective drugs that have been applied in AMD treatment, taking the activation or inhibition interactions of the 5 hub genes into account, we may combine different drugs for targeting different genes of the five to achieve the effects of antineovascularization, improving autophagy function, alleviating cellular senescence, and inhibiting the inflammatory response. For the 13 drugs that have not been applied in AMD, further study is needed to verify whether they could be used for AMD treatment safely and effectively before being combined with other drugs and whether the benefits of these suggested drugs for AMD are greater than the adverse effects.

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